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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

**ATTORNEY DOCKET NO. 065691-0265**

Applicant: Alain MOUSSY et al.  
Title: METHOD FOR SCREENING COMPOUNDS CAPABLE OF  
DEPLETING MAST CELLS (As Amended)  
Appln. No.: 10/022,842  
Filing Date: December 20, 2001  
Examiner: Unassigned  
Art Unit: 1632

**PRELIMINARY AMENDMENT**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the present Application, Applicants respectfully request that the above-identified application be amended as follows:

**IN THE TITLE:**

On page 1, please delete the title and replace it with the following:

METHOD FOR SCREENING COMPOUNDS CAPABLE OF DEPLETING MAST CELLS

**IN THE SPECIFICATION:**

On page 6, delete lines 1-3, and replace them with the following in accordance with 37 C.F.R. §1.121. A marked up version showing the changes is attached:

- 5'AAGAAGAGATGGTACCTCGAGGGGTGACCC3' (SEQ ID No1) sens
- 5'CTGCTTCGCGGCCGCGTAACTCTTCTCAACCA3' (SEQ ID No2) antisens

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**IN THE CLAIMS:**

Please cancel claim 14 without prejudice or disclaimer.

In accordance with 37 C.F.R. §1.121, please substitute for original claims 3-13, 15 and 17 the following rewritten version of the same claims, as amended. The changes are shown explicitly in the attached "Version with Markings to Show Changes Made."

3. (Amended) A method according to claim 1, wherein mast cells are chosen from isolated mast cells and cell lines derived thereof, BaF3, IC-2 mouse cells, HMC-1, P815 available at ATCC under the accession number TIB-64, 10P2 available at ATCC under the accession number CRL-2034, 10P12 available at ATCC under the accession number CRL-2036, 11P0-1 available at ATCC under the accession number CRL-2037, and cell lines derived thereof.

4. (Amended) A method according to claim 1, wherein other hematopoietic cells that are not mast cells or related cells or cell lines are selected from the group consisting of human T lymphocyte Jurkat cell line (ATCC N° TIB-152 and cell lines derived thereof), the human B lymphocyte Daudi or Raji cell line (ATCC N° CCL-213 and CCL-86 respectively and cell lines derived thereof), the human monocytic U 937 cell line (ATCC N° CRL-1593.2) and the human HL-60 cell line (ATCC N° CCL-240), cell lines derived thereof ATCC N° CRL-2258 and CRL-2392) and normal human CD34+ cells that are expanded in a culture medium comprising a cocktail of cytokine except SCF.

5. (Amended) A method according to claim 1, wherein compounds capable of depleting specifically mast cells at a concentration below 10  $\mu$ M, preferably below 1  $\mu$ M are selected.

6. (Amended) A method according to claim 1, wherein the compounds exhibiting Ratios E/S ranging from 1/1000 to 1/5 are selected.

7. (Amended) A method according to claim 1, wherein the cell death assay further comprises a cell proliferation assay, a cell viability assay and/or an apoptosis assay.

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8. (Amended) A method according to claim 1, wherein the extent of cell death is measured by <sup>3</sup>H thymidine incorporation, the trypan blue exclusion method, using propidium iodide or by the <sup>51</sup>Cr-release assay.

9. (Amended) A method according to claim 1, wherein the extent of cell death is determined by a test of intracellular esterase activity, and a test of plasma membrane integrity, preferably using fluorescent calcein and ethidium homodimer-1.

10. (Amended) A method according to claim 1, wherein the extent of cell death is determined by discriminating between living and dead cells using DiOC<sub>18</sub> and propidium iodide.

11. (Amended) A method according to claim 1, wherein the extent of cell death is measured by fluorometric assays of cell viability and cytotoxicity using a fluorescence microscope, a fluorometer, a fluorescence microplate reader or a flow cytometer.

12. (Amended) A method according to claim 1, wherein the mast cells that are IL-3 dependent cells are cultured in a culture media comprising IL-3 at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

13. (Amended) A method according to claim 1, wherein compounds to be tested are selected from inhibitors of tyrosine kinases, such as Akt, c-Cbl, CRKL, Doc, p125 Fak, Fyn, Grap, Jak2, Lyn, MAPK, MATK, PI3-K, PLC- $\gamma$ , Raf1, Ras, SHP-1, SHP2 (Syp), Tec, Vav and Flt-3.

15. (Amended) A compound obtainable by the method according to claim 1, wherein said compound is capable of depleting mast cells and has no significant toxicity for other hematopoietic cells, preferably compounds having an E/S ratio ranging 1/1000 to 1/5.

17. (Amended) A method for treating a disease selected from autoimmune diseases, allergic diseases, bone loss, tumor angiogenesis, inflammatory diseases, inflammatory bowel diseases (IBD), interstitial cystitis, mastocytosis, infections diseases, and CNS disorders comprising administering a compound obtainable from a method according to claim 1 to a mammal in need of such treatment.

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25. (Amended) A method according to claim 23, wherein the compound is administered with aerosolized formulations to target areas of a patient's respiratory tract, intranasal or topical formulation.

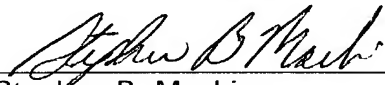
34. (Amended) A method according to claim 29 comprising further administering at least one antibiotic selected bacitracin, the cephalosporins, the penicillins, the aminoglycosides, the tetracyclines, the streptomycins and the macrolide antibiotics such as erythromycin; the fluoroquinolones, actinomycin, the sulfonamides and trimethoprim.

#### REMARKS

Applicants respectfully request that the foregoing amendments be made prior to examination of the present application.

Respectfully submitted,

July 29, 2002  
Date

  
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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE TITLE:**

[METHOD FOR IDENTIFYING COMPOUNDS THAT SPECIFICALLY DEplete  
MAST CELLS] METHOD FOR SCREENING COMPOUNDS CAPABLE OF DEPLETING  
MAST CELLS

**IN THE SPECIFICATION:**

- 5'AAGAAGAGATGGTACCTCGAGGGGTGACCC3' [(SEQ ID No2)] (SEQ ID  
No1) sens  
- 5'CTGCTTCGCGGCCGCGTTAACTCTTCTCAACCA3' [(SEQ ID No3)]  
(SEQ ID No2) antisens

**IN THE CLAIMS:**

3. (Amended) A method according to claim 1 [or 2], wherein mast cells are chosen from isolated mast cells and cell lines derived thereof, BaF3, IC-2 mouse cells, HMC-1, P815 available at ATCC under the accession number TIB-64, 10P2 available at ATCC under the accession number CRL-2034, 10P12 available at ATCC under the accession number CRL-2036, 11P0-1 available at ATCC under the accession number CRL-2037, and cell lines derived thereof.

4. (Amended) A method according to [one of claims 1 to 3] claim 1, wherein other hematopoietic cells that are not mast cells or related cells or cell lines are selected from the group consisting of human T lymphocyte Jurkat cell line (ATCC N° TIB-152 and cell lines derived thereof), the human B lymphocyte Daudi or Raji cell line (ATCC N° CCL-213 and CCL-86 respectively and cell lines derived thereof), the human monocytic U 937 cell line (ATCC N° CRL-1593.2) and the human HL-60 cell line (ATCC N° CCL-240), cell lines derived thereof ATCC N° CRL-2258 and CRL-2392) and normal human CD34 + cells that are expanded in a culture medium comprising a cocktail of cytokine except SCF.

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5. (Amended) A method according to [one of claims 1 to 4] claim 1, wherein compounds capable of depleting specifically mast cells at a concentration below 10  $\mu$ M, preferably below 1  $\mu$ M are selected.

6. (Amended) A method according to [one of claims 1 to 5] claim 1, wherein the compounds exhibiting Ratios E/S ranging from 1/1000 to 1/5 are selected.

7. (Amended) A method according to [one of claims 1 to 6] claim 1, wherein the cell death assay further comprises a cell proliferation assay, a cell viability assay and/or an apoptosis assay.

8. (Amended) A method according to [one of claims 1 to 6] claim 1, wherein the extent of cell death is measured by 3H thymidine incorporation, the trypan blue exclusion method, using propidium iodide or by the  $^{51}\text{Cr}$ -release assay.

9. (Amended) A method according to [one of claims 1 to 6] claim 1, wherein the extent of cell death is determined by a test of intracellular esterase activity, and a test of plasma membrane integrity, preferably using fluorescent calcein and ethidium homodimer-1.

10. (Amended) A method according to [one of claims 1 to 6] claim 1, wherein the extent of cell death is determined by discriminating between living and dead cells using DiOC<sub>18</sub> and propidium iodide.

11. (Amended) A method according to [one of claims 1 to 10] claim 1, wherein the extent of cell death is measured by fluorometric assays of cell viability and cytotoxicity using a fluorescence microscope, a fluorometer, a fluorescence microplate reader or a flow cytometer.

12. (Amended) A method according to [one of claims 1 to 11] claim 1, wherein the mast cells that are IL-3 dependent cells are cultured in a culture media comprising IL-3 at a concentration comprised between 0.5 and 10 ng/ml, preferably between 1 to 5 ng/ml.

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13. (Amended) A method according to [one of claims 1 to 12] claim 1, wherein compounds to be tested are selected from inhibitors of tyrosine kinases, such as Akt, c-Cbl, CRKL, Doc, p125 Fak, Fyn, Grap, Jak2, Lyn, MAPK, MATK, PI3-K, PLC- $\gamma$ , Raf1, Ras, SHP-1, SHP2 (Syp), Tec, Vav and Flt-3.

15. (Amended) A compound obtainable by the method according to [one of claims 1 to 12] claim 1, wherein said compound is capable of depleting mast cells and has no significant toxicity for other hematopoietic cells, preferably compounds having an E/S ratio ranging 1/1000 to 1/5.

17. (Amended) A method for treating a disease selected from autoimmune diseases, allergic diseases, bone loss, tumor angiogenesis, inflammatory diseases, inflammatory bowel diseases (IBD), interstitial cystitis, mastocytosis, infections diseases, and CNS disorders comprising administering a compound obtainable from a method according to [one of claims 1 to 14] claim 1 to a mammal in need of such treatment.

25. (Amended) A method according to [one of claims 23 and 24] claim 23, wherein the compound is administered with aerosolized formulations to target areas of a patient's respiratory tract, intranasal or topical formulation.

34. (Amended) A method according to claim 29 [or 30] comprising further administering at least one antibiotic selected bacitracin, the cephalosporins, the penicillins, the aminoglycosides, the tetracyclines, the streptomycins and the macrolide antibiotics such as erythromycin; the fluoroquinolones, actinomycin, the sulfonamides and trimethoprim.